Flow Cytometric Assessment of MRD

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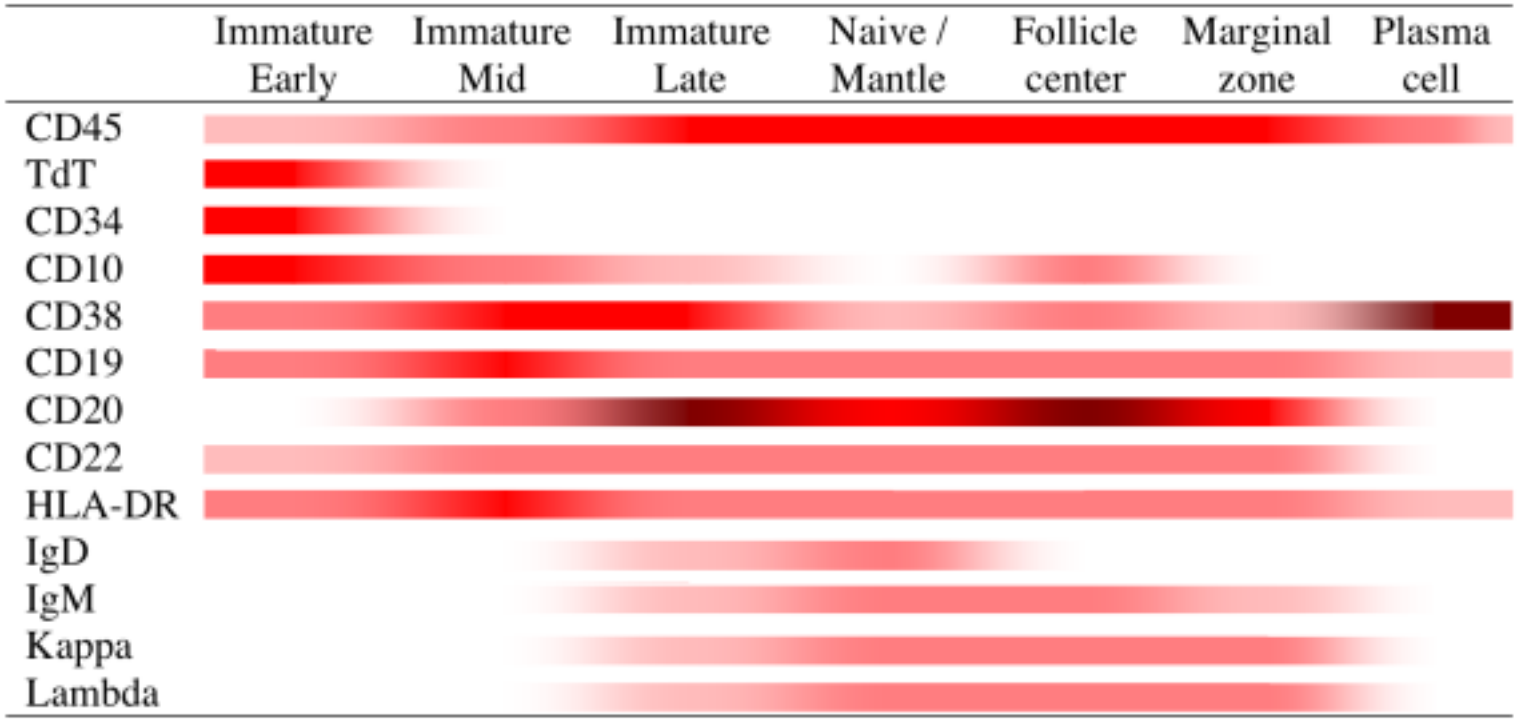
Identification

Abnormal population identification

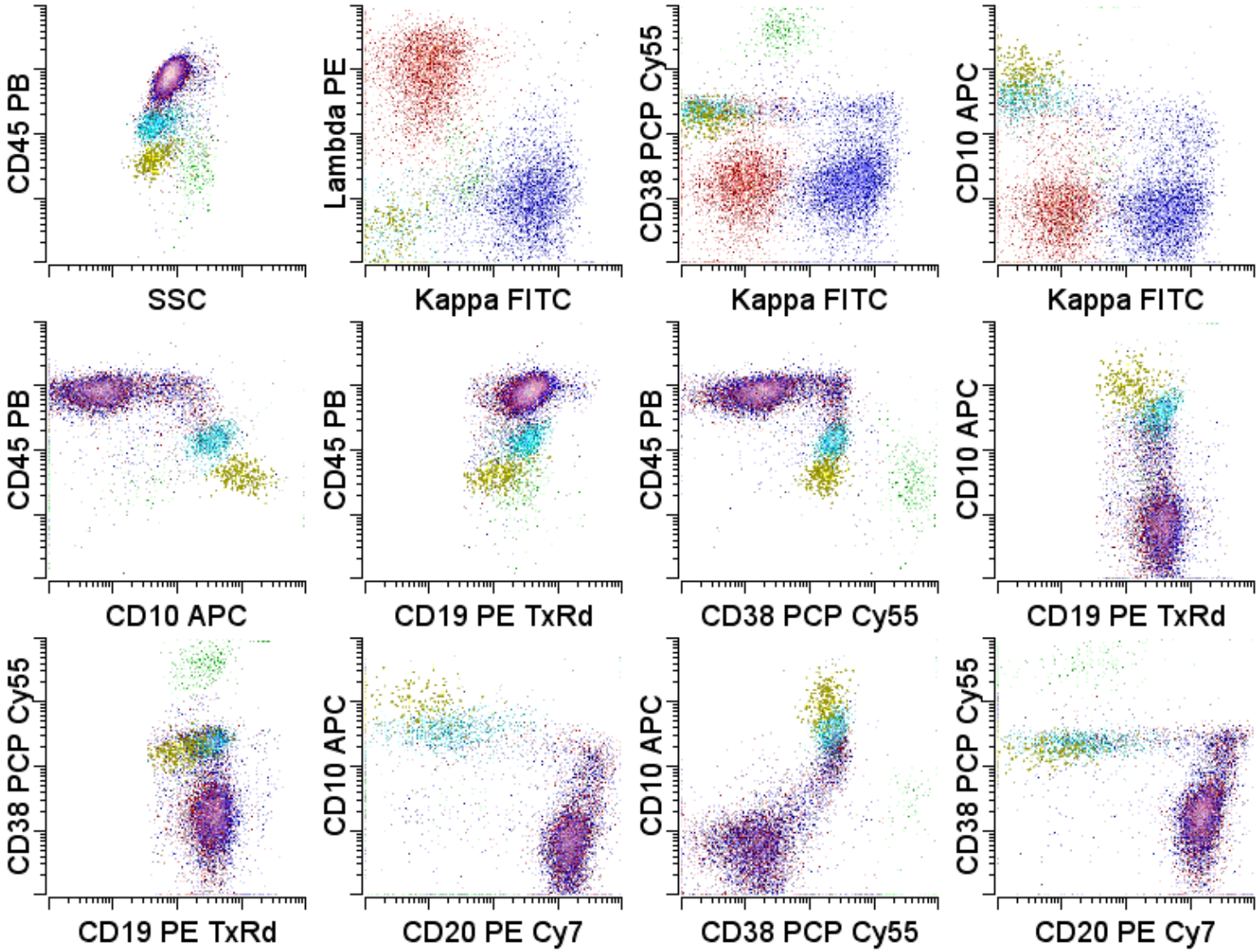
- Normal
  - Antigens expressed in consistent and reproducible patterns with maturation
- Neoplastic
  - Increased or decreased normal antigens
  - Asynchronous maturational expression
  - Aberrant antigen expression
  - Homogeneous expression

Normal B cell Maturation



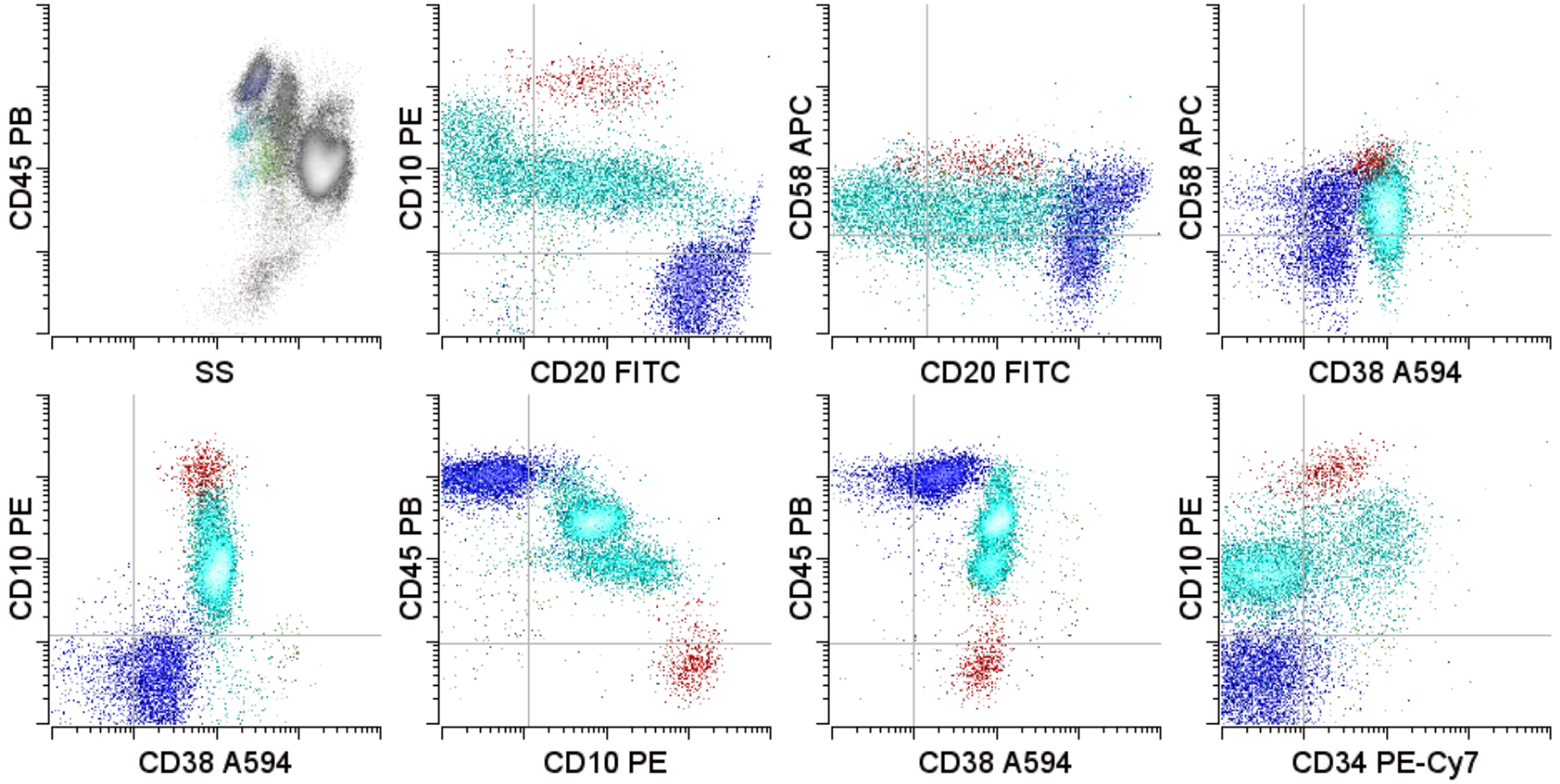
Wood and Borowitz (2011) Henry's Laboratory Medicine

Normal B cell Maturation



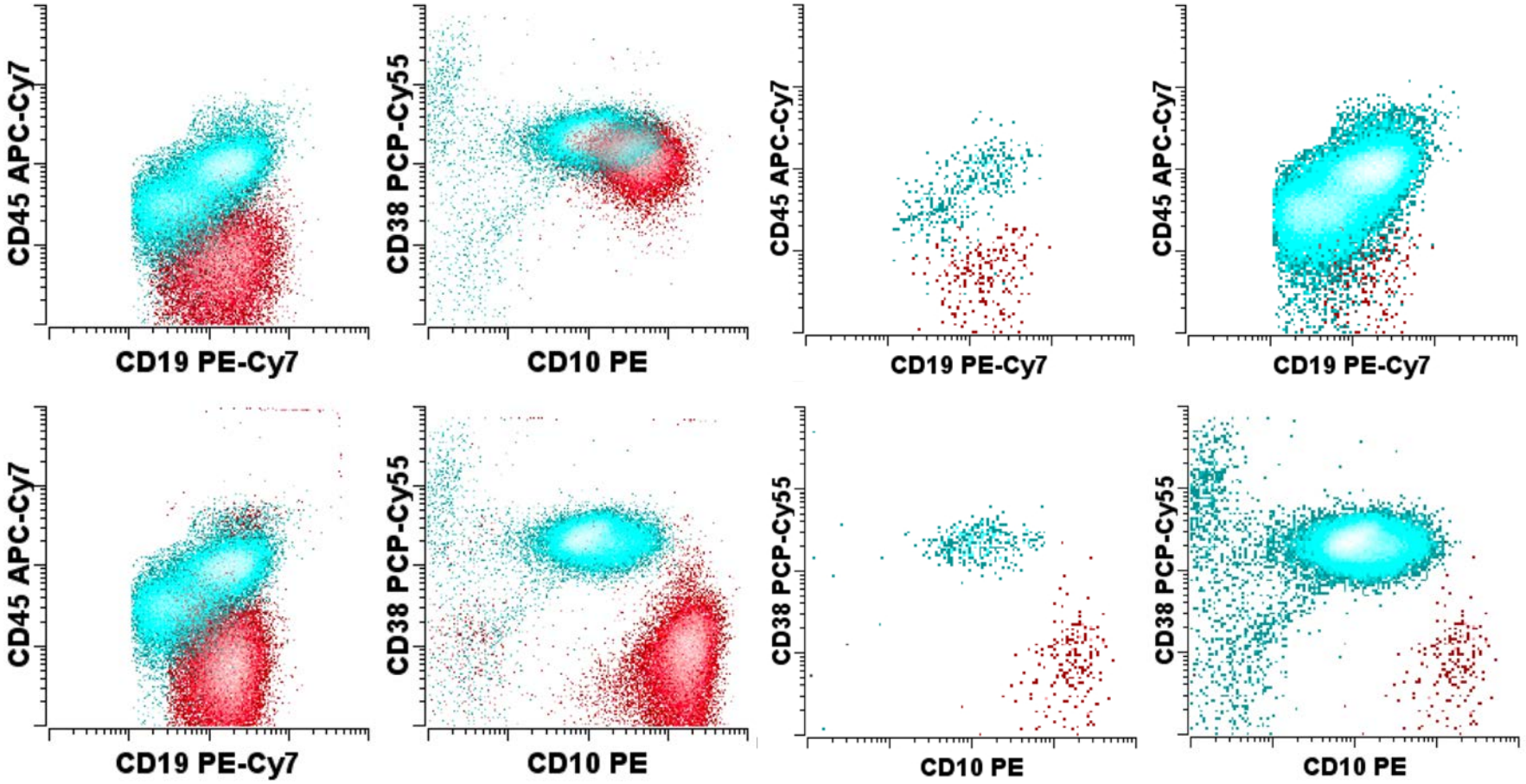
Wood (2004) Methods Cell Biology 75:559-576

ALL MRD



0.1% abnormal immature B cells

Informative Immunophenotype



ALL Informative Antigens

Table 1. Useful antibody combinations for immunophenotypic MRD detection.

*Antibody combinations**	*Suitable cases* (n)	(%)	*LAIP frequency in childhood ALL°*
CD38/CD10/CD34/CD19	36/63	57.1%	30-50%
CD45/CD10/CD34/CD19	17/64	26.6%	30-50%
CD21/CD10/CD34/CD19	0/57	0	5-10%
CD22/CD10/CD34/CD19	0/55	0	20-30%
CD58/CD10/CD34/CD19	11/62	17.7%	40-60%
TdT/CD10/CD34/CD19	22/61	36.1%	30-50%
CD13/CD10/CD34/CD19	8/62	12.9%	10-20%
CD15/CD10/CD34/CD19	3/58	5.2%	5-10%
CD33/CD10/CD34/CD19	2/59	3.4%	5-10%
CD65/CD10/CD34/CD19	0/59	0	5-10%
CD66c/CD10/CD34/CD19	15/62	24.2%	10-20%
CD10/NG2/CD34/CD19	9/60	15.0%	3-5%
CD10/CD56/CD34/CD19	3/57	5.3%	5-10%

**FITC/PE/PerCP/APC; °Refs. 2,3,5,8.*

From Krampera et al (2006) Haematologica 91:1109-1112

Minimal MRD Reagents

- Simple two-tube panel
  - CD10 FITC / CD20 PE / CD45 PerCP / CD19 APC
  - CD34 FITC / CD9 PE / CD45 PerCP / CD19 APC

- Abnormalities detected (N=82)
  - Tube 1 = 93% of cases
  - Tube 2 = 94% of cases
  - Tubes 1 and 2 combined = 99%

Weir, et al (1999) Leukemia 13:558-567

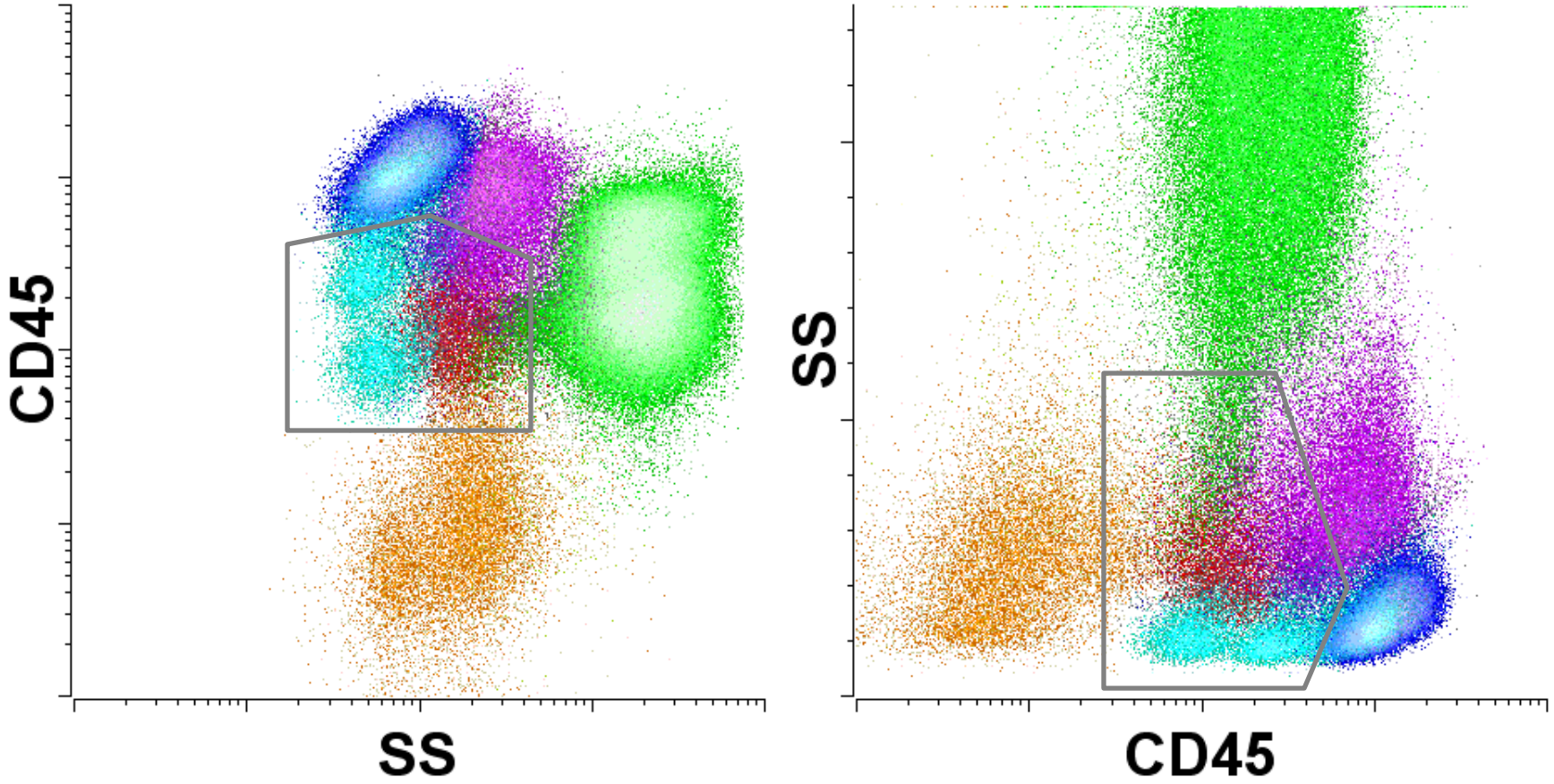
Risk Adapted Therapy - AALL03B1

- 6 color flow cytometric assay

	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
Tube A	CD20	CD10	CD38	CD19	CD58	CD45
Tube B	CD9	CD13/33	CD34	CD19	CD10	CD45
Tube D	Syto16		CD3	CD19	CD71	CD45

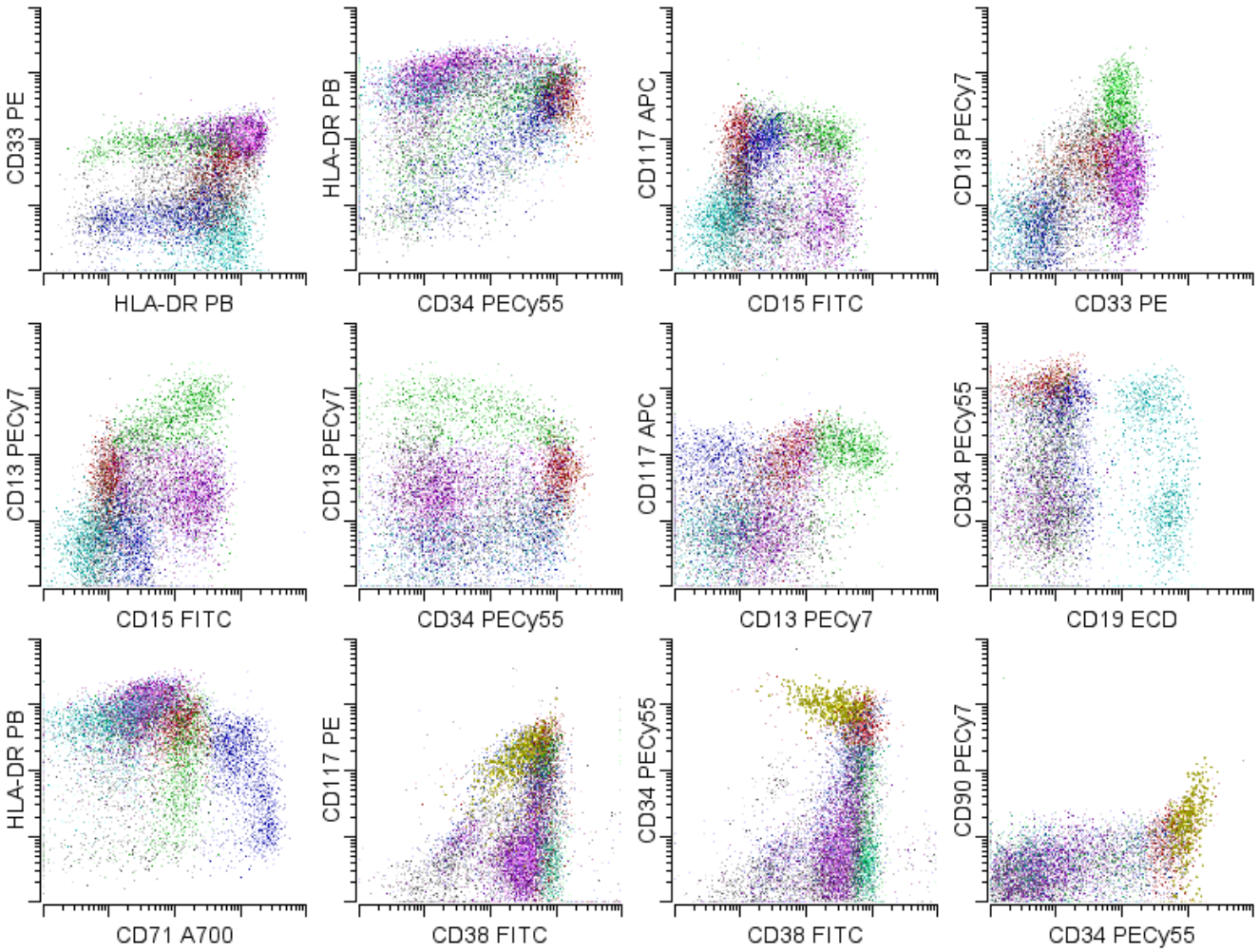
- Collect 750,000 events

CD45/SS



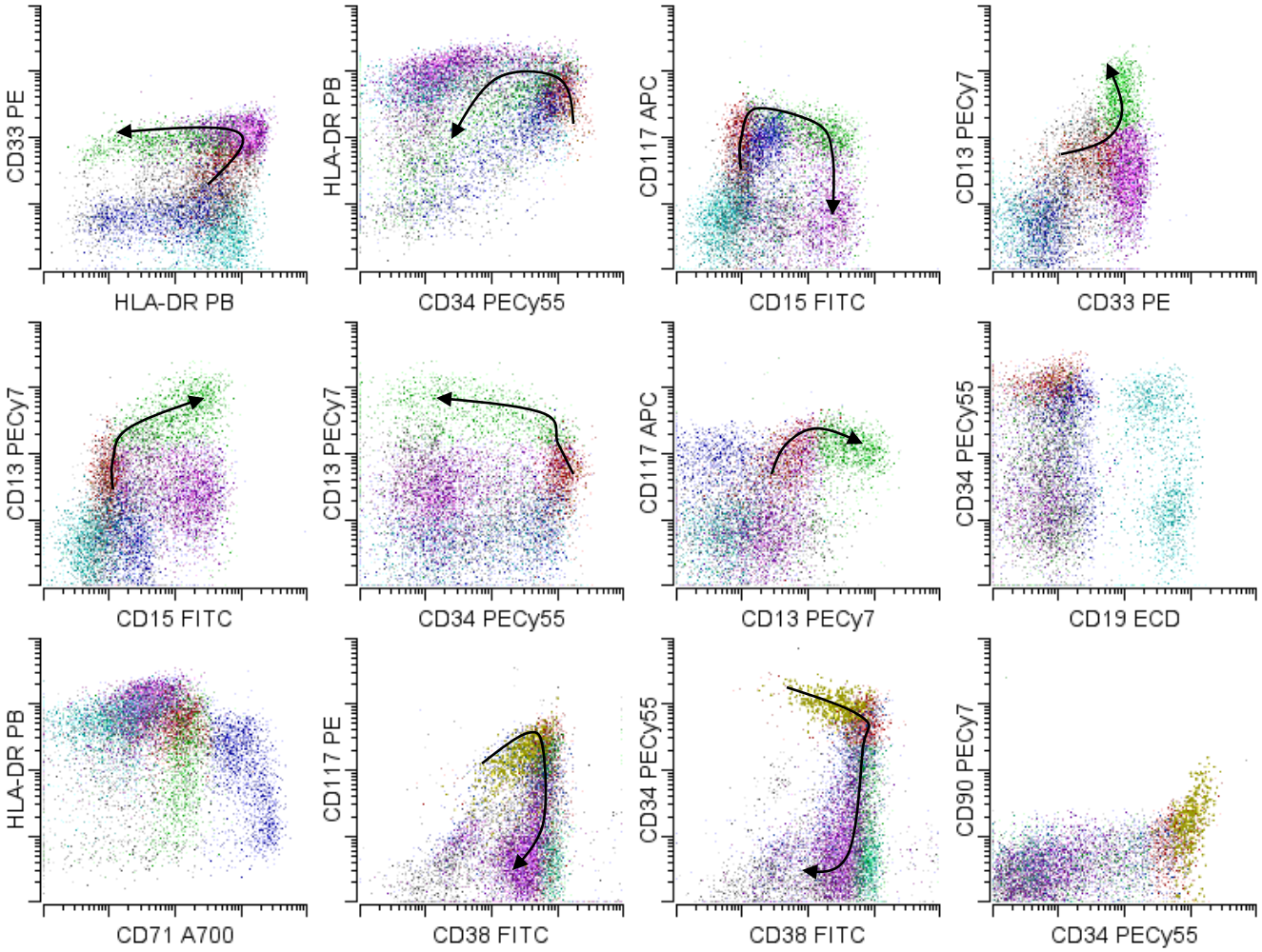
Borowitz et al (1993) AJCP 100:534-40.
Steltzer et al (1993) Ann NY Acad Sci 667:265-280

Normal Blast Maturation



Wood (2004) Methods Cell Biology 75:559-576

Normal Granulocyte Maturation



AML Informative Antigens

Table 2
Distribution of LAIP and of LAIP classes in 1400 patients with newly diagnosed and untreated AML (Laboratory for Leukemia Diagnostics, Munich, Germany)

LAIP class	LAIP	*n*	%
Asynchronous	Total	652	20.6
	CD11b+CD117+CD34−	156	4.9
	CD11b+CD117+CD34+	92	2.9
	CD11b+CD117−CD34+	36	1.1
	CD34+CD116+CD33+	113	3.6
	CD34+CD15+CD33+	193	6.1
	CD65+CD87+CD34+	12	0.4
	CD65+CD87−CD34+	50	1.6
Cross-lineage	Total	742	23.5
	CD34+CD13+CD19+	48	1.5
	CD34+CD2+CD33+	51	1.6
	CD34+CD56+CD33+	83	2.6
	CD34−CD13+CD19+	21	0.7
	CD34−CD2+CD33+	33	1.0
	CD34−CD56+CD33+	189	6.0
	CD4+CD13+CD14−	87	2.8
	CD7+CD33+CD34−	75	2.4
	CD7+CD33+CD34+	155	4.9
Lack of expression	Total	625	19.8
	CD15+CD13+CD33−	6	0.2
	CD15+CD13−CD33+	7	0.2
	CD34−CD135+CD117+	17	0.5
	CD38−CD133+CD34+	10	0.3
	CD4+CD13−CD14+	7	0.2
	CD9−CD34+CD33+	30	0.9
	CD9−CD34−CD33+	34	1.1
	HLA-DR+CD33−CD34+	12	0.4
	HLA-DR−CD33+CD34−	143	4.5
	HLA-DR−CD33+CD34+	37	1.2
	MPO+LF−cCD15−	315	10.0
	MPO+LF−cCD15+	4	0.1
	MPO−LF+cCD15+	3	0.1
Overexpression	Total	1139	36.1
	CD11b−CD117++CD34+	9	0.3
	CD13++CD34++	163	5.2
	CD15++CD13++CD33++	52	1.6
	CD34++CD135+CD117++	35	1.1
	CD34++CD33++	65	2.1
	CD34−7.1++CD33+	53	1.7
	CD36++CD235a++CD45(+)	25	0.8
	CD38++CD133++CD34++	16	0.5
	CD4++CD64++CD45++	144	4.6
	CD4+CD13++CD14++	19	0.6
	CD61++CD14−CD45+	5	0.2
	CD65++CD87++	162	5.1
	CD90++CD117++CD34+	23	0.7
	HLA-DR++CD33++CD34++	41	1.3
	TdT(+)cCD33++cCD45++	327	10.4
Total		3158	100.0

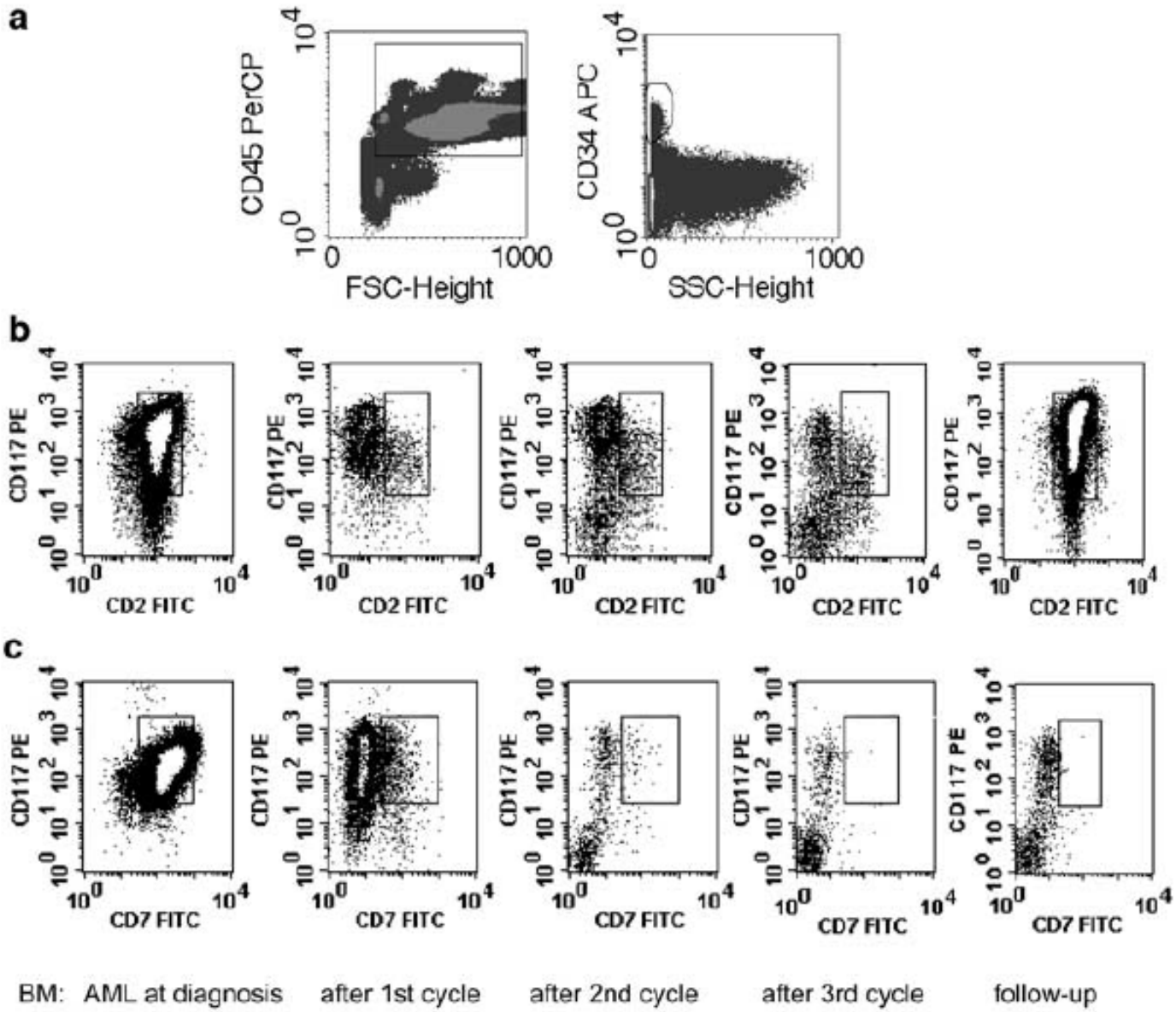
Average 2.3 LAIP per patient

From Kern et al (2005) CRC Rev Onc/Hem 56:283-309

Population Identification

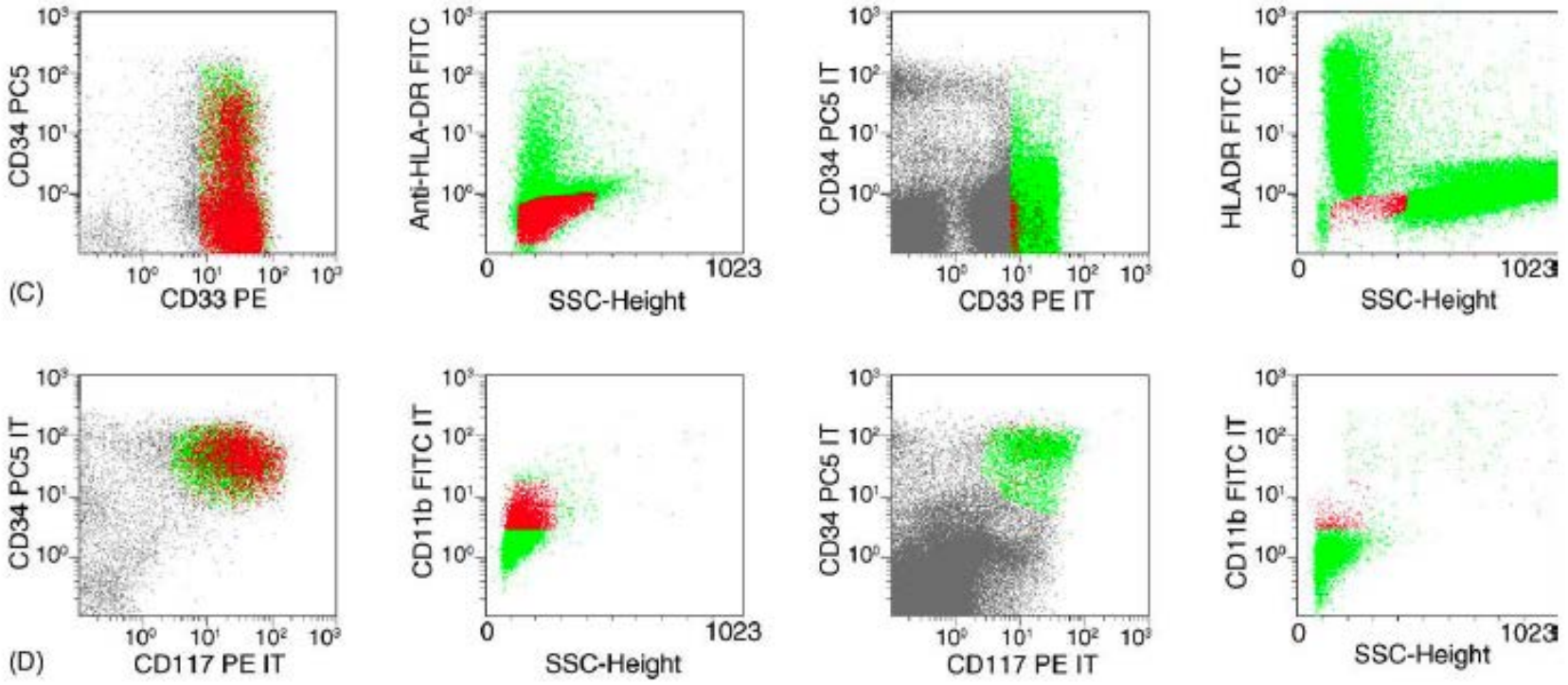
- Leukemia-Associated immunophenotype (LAIP)
  - At diagnosis
    - Immunophenotypic deviation relative to normal is identified
    - Informative reagent combinations selected (reduced or custom set)
    - Gate is created for monitoring
  - Follow-up
    - Run reduced reagent combination
    - Count events in pre-defined gate

- Deviation from normal maturation
  - At diagnosis
    - Immunophenotypic deviation relative to normal counterpart is identified
    - Uniform reagent combinations utilized
  - Follow-up
    - Identify discrete population having immunophenotype different than normal
    - Use diagnostic immunophenotype as starting point



From Feller et al (2004) Leukemia 18:1380-1390

LAIP

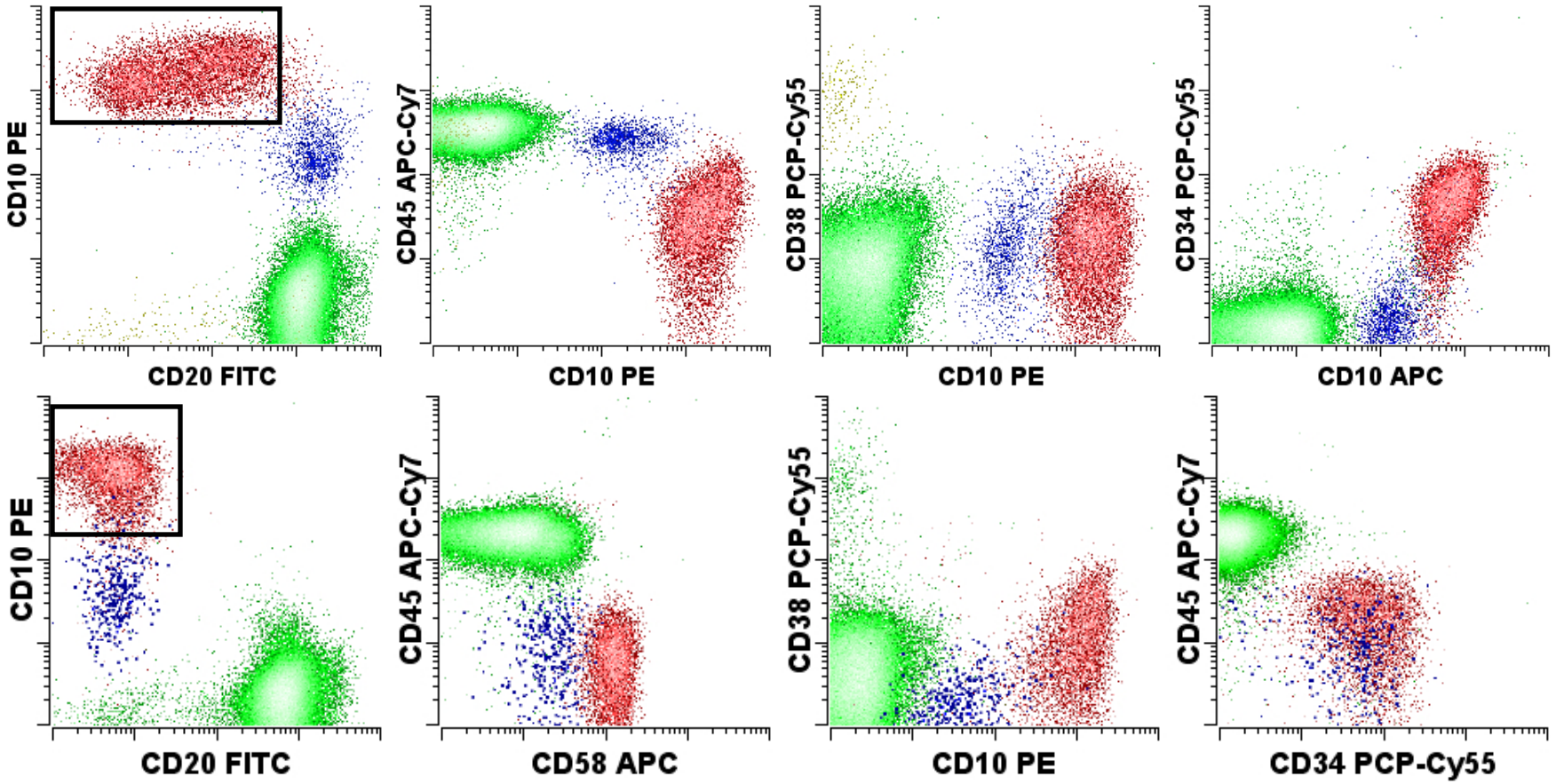


Focus is immunophenotype, not population

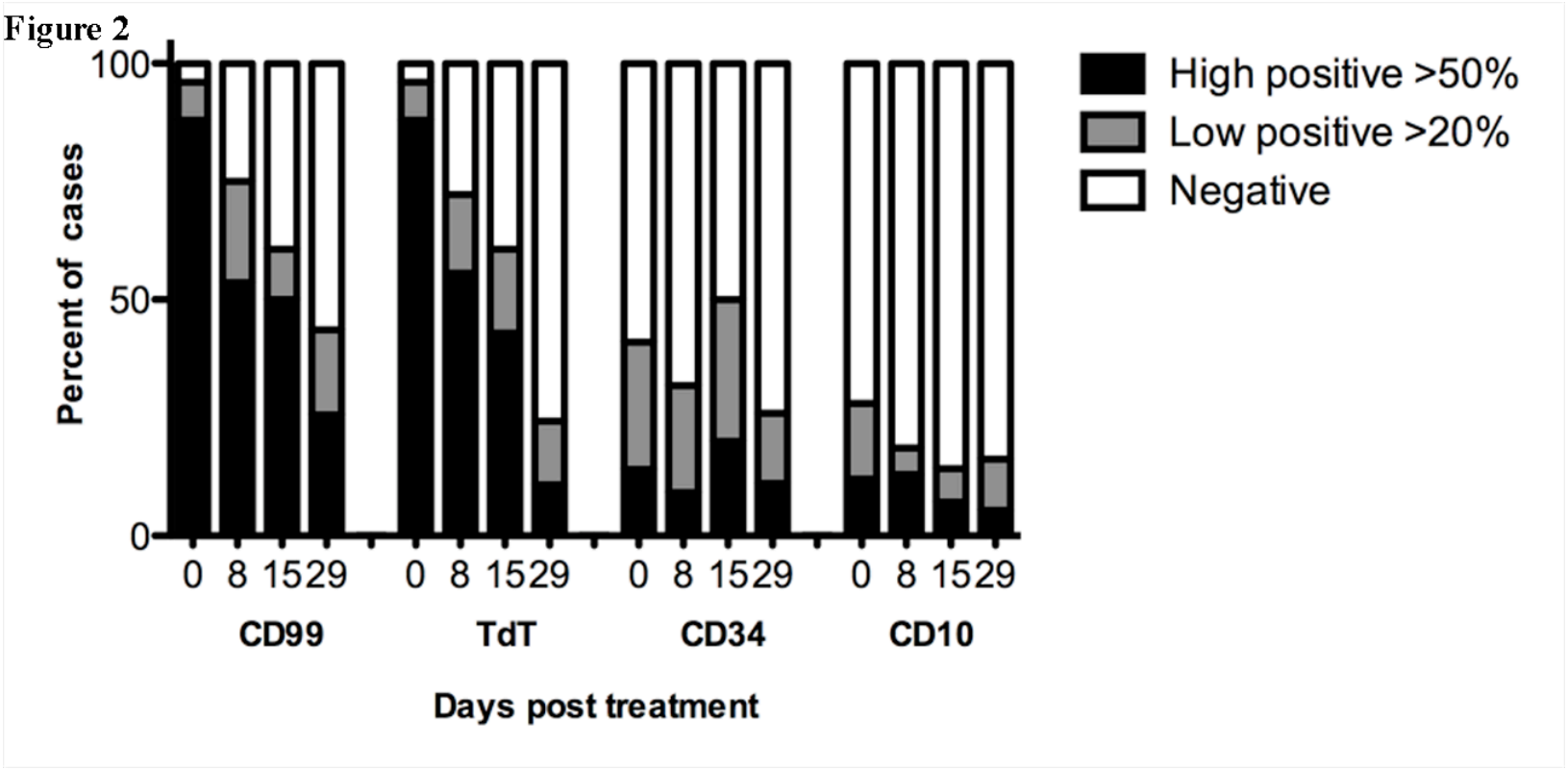
From Kern et al (2005) CRC Rev Onc/Hem 56:283-309

Immunophenotypic Stability

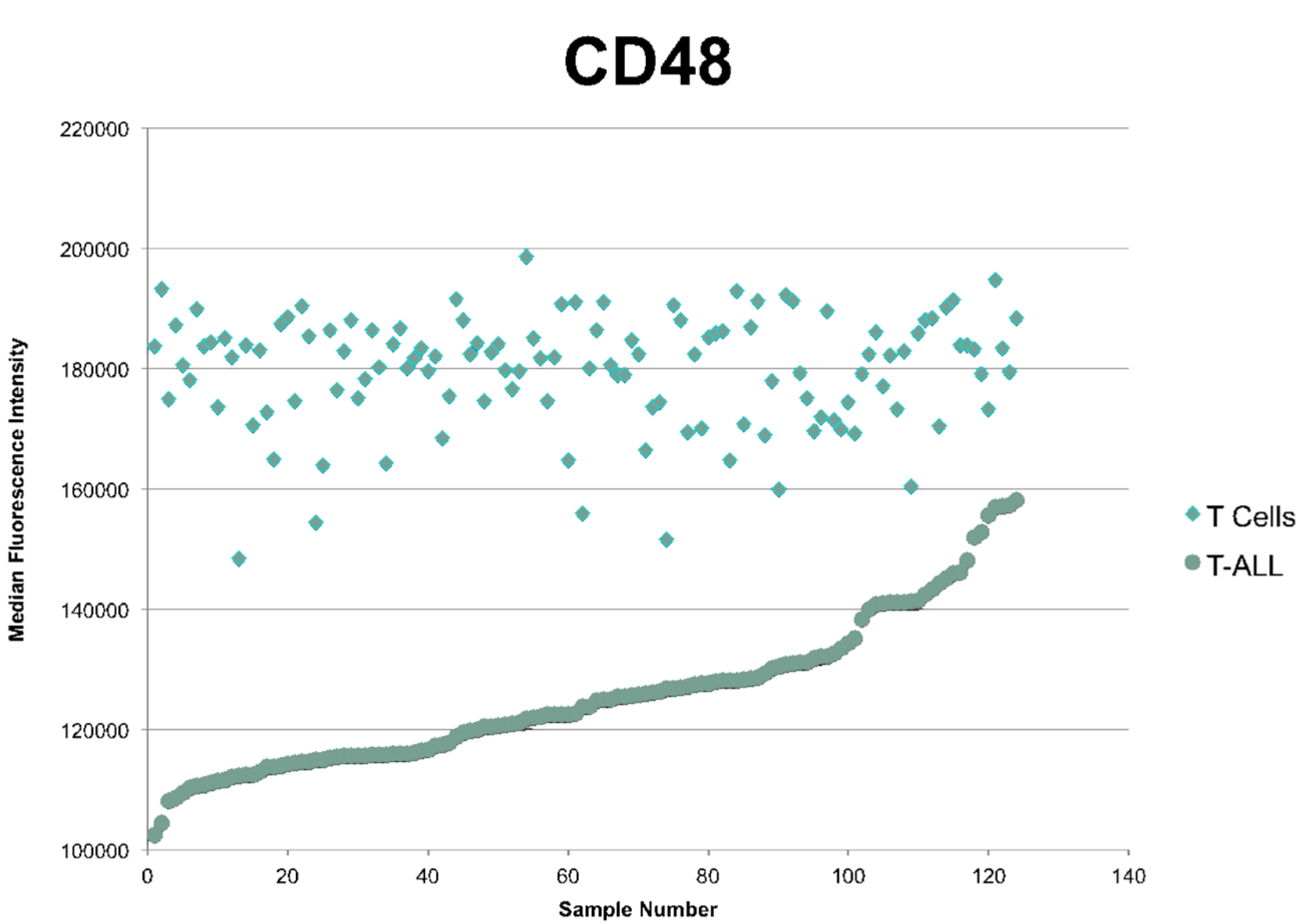
- ALL



Immunophenotypic Stability T-ALL

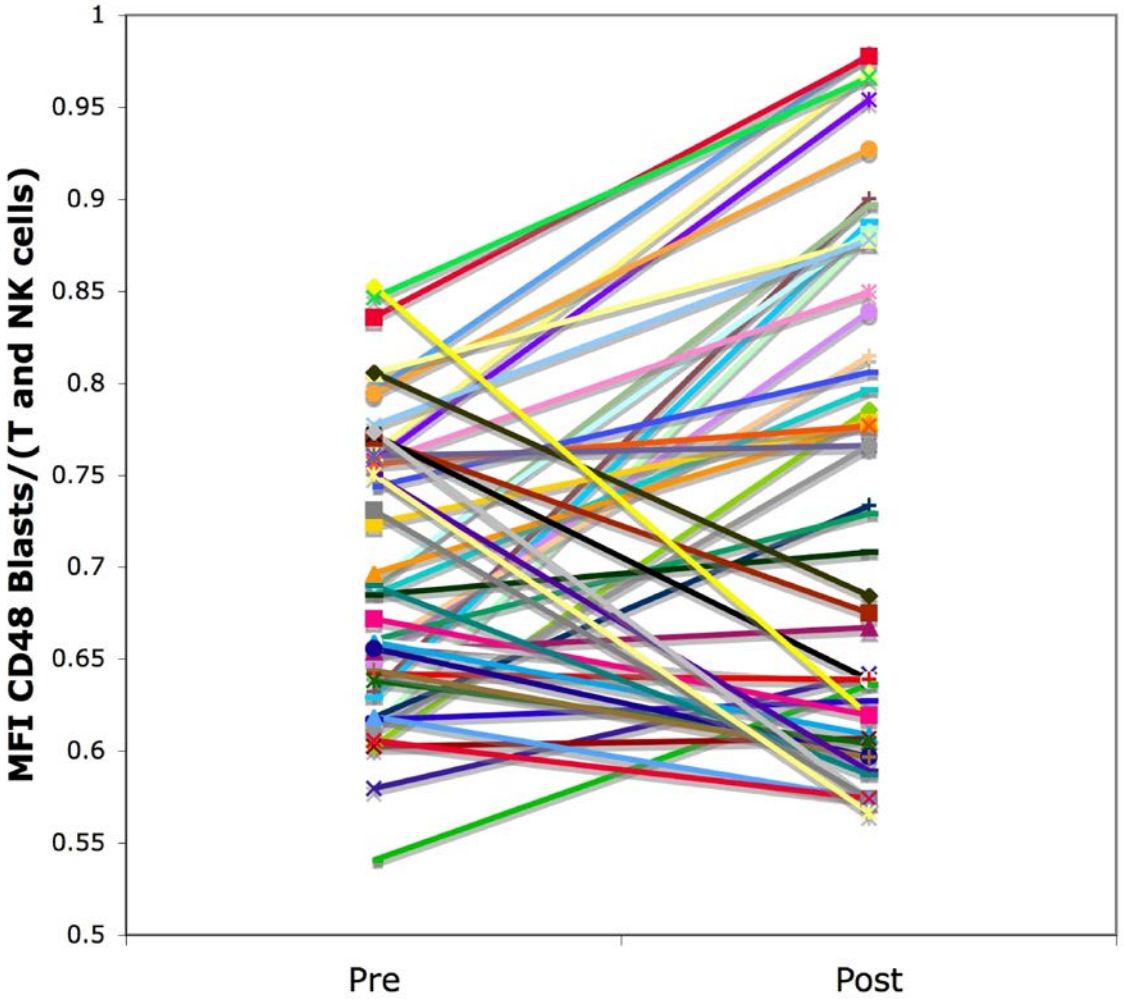


Roshal, et al (2010) Cytometry 78B:139-146

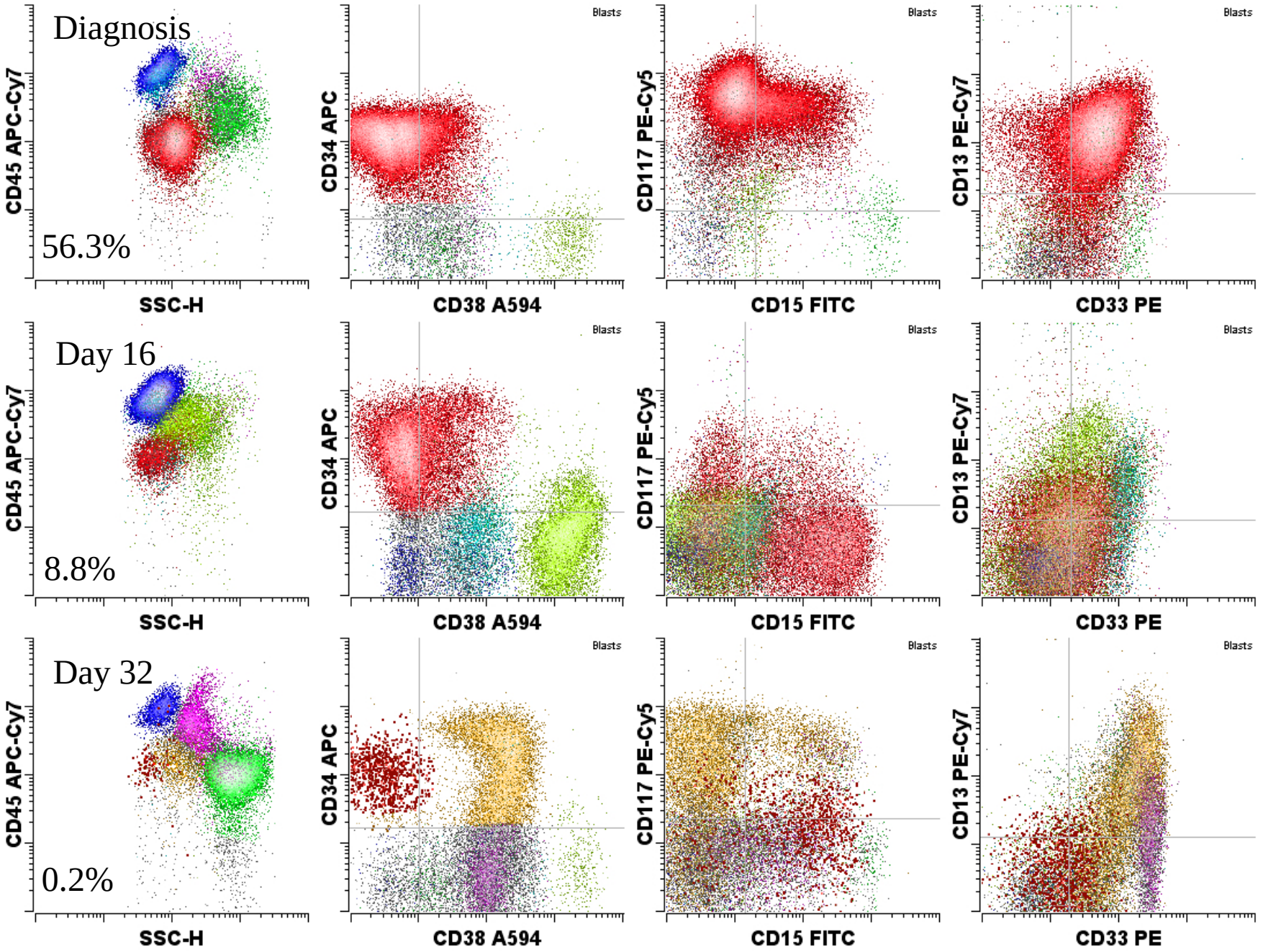


Wood, et al (2011) ASH abstract

CD48 MRD



Wood, et al (2011) ASH abstract


Diagnosis
56.3%
Day 16
8.8%
Day 32
0.2%
CD45 APC-Cy7
SSC-H
CD34 APC
CD38 A594
CD117 PE-Cy5
CD15 FITC
CD13 PE-Cy7
CD33 PE
Blasts

HP06-05604

Population Identification

- Leukemia-Associated immunophenotype (LAIP)
  - Advantages
    - Conceptually simple and objective
    - Reduced reagent expense for follow up
  - Disadvantages
    - Requires pre-treatment sample to define LAIP
    - Requires immunophenotypic stability
    - Any event in pre-defined gate regarded as MRD

- Deviation from normal maturation
  - Advantages
    - Does not require pretreatment sample
    - Uniform reagent combinations utilized
    - Improved specificity through population identification
    - Less sensitive to immunophenotypic instability
  - Disadvantages
    - Requires detailed immunophenotypic knowledge (expert)
    - Subjective
    - More time consuming

Timing

- Induction nadir (day 14)
  - Reduced background populations
  - Hypoplastic with many apoptotic cells
- End of induction
  - ALL - Few immature B cells
  - AML - Active marrow regeneration, increased precursors
- End of consolidation
  - ALL - Larger number of immature B cells
  - AML - Normal marrow populations

Enumeration

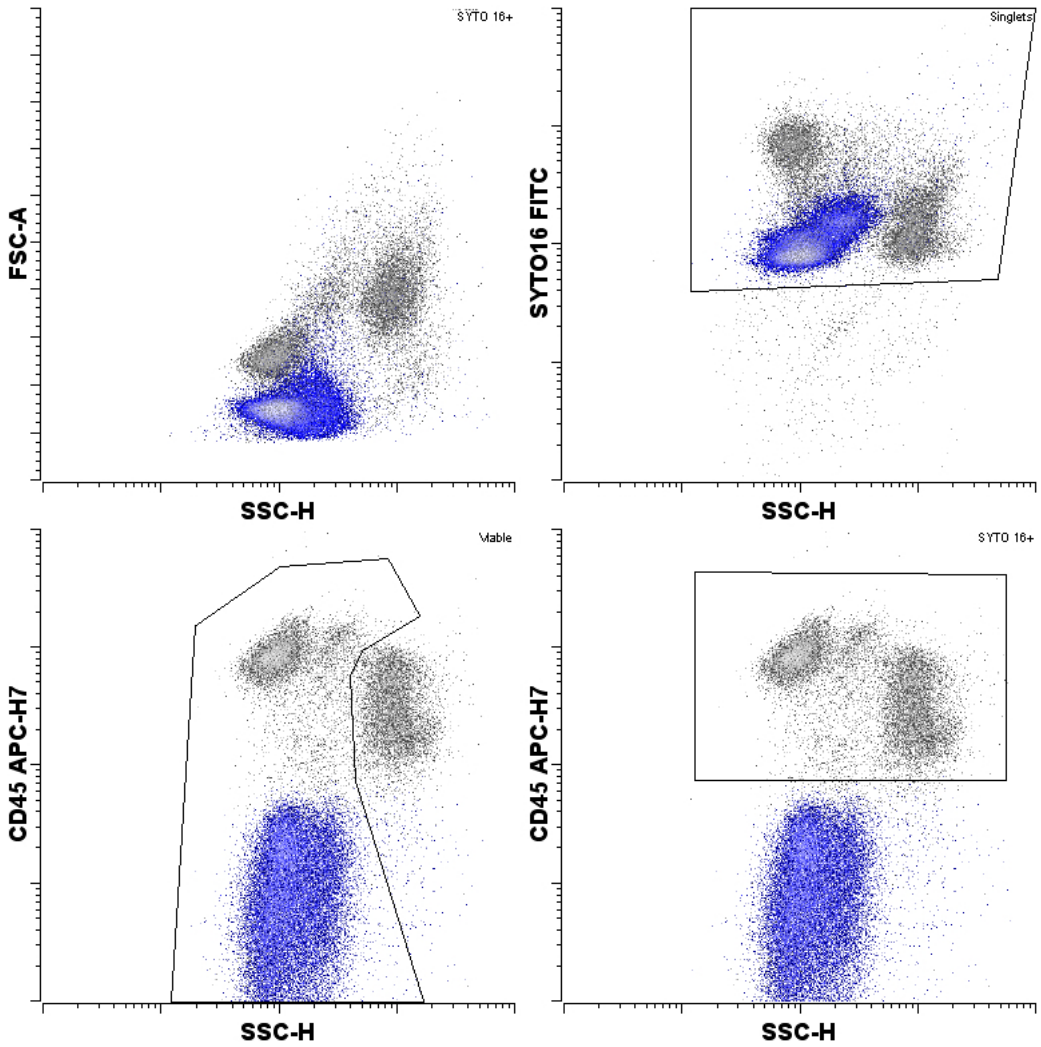
Sample Acquisition

- Identification
  - Distinguish normal from abnormal
    - Degree of immunophenotypic aberrancy
    - Number and immunophenotype of background populations
  - How many events define a population?
    - 10-50 events
- Enumeration
  - Requires complete discrimination of population
    - Insufficiently informative immunophenotype
    - Maturational expression
  - Reproducibility (Poisson counting statistics)
    - CV ~ Sqrt (N)/N
      - 100 events gives CV of 10%
    - Sensitivity of 0.01% requires 1,000,000 events

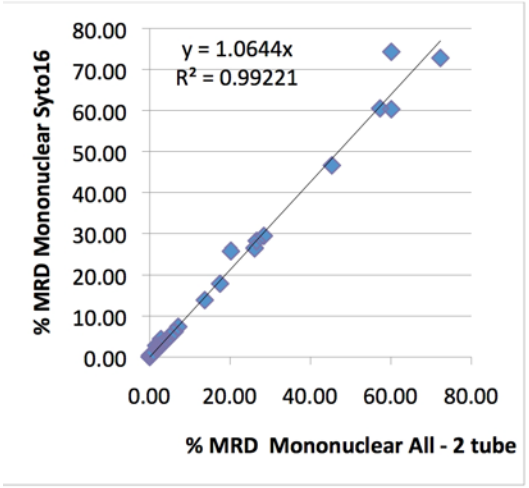
Denominator

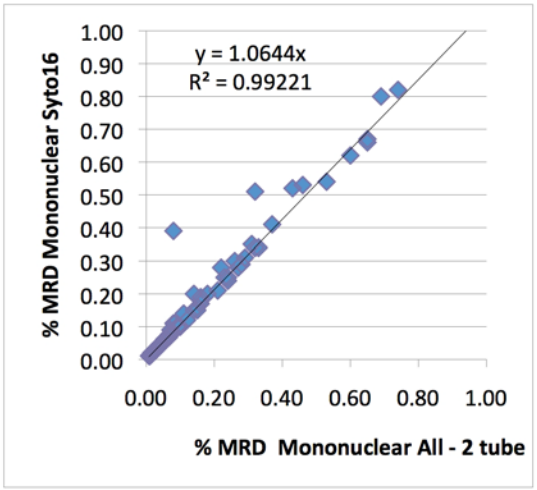
- Total nucleated cells
  - Most comparable to morphology
  - DNA binding dye often used (Syto16, Draq5, etc.)
    - Incomplete RBC lysis, platelet aggregates
  - Under-representation of NRBCs with lysis and washing
- White cells
  - CD45 positive cells + neoplasm
  - Variable CD45 on early NRBCs
  - Overestimation with erythroid hyperplasia
- Mononuclear cells
  - Exclude granulocytic lineage (high side scatter)
  - Most comparable to Ficoll-prepared samples
    - Early MRD literature used Ficoll
  - Reduced variability due to granulocytic degeneration
    - Shipped samples

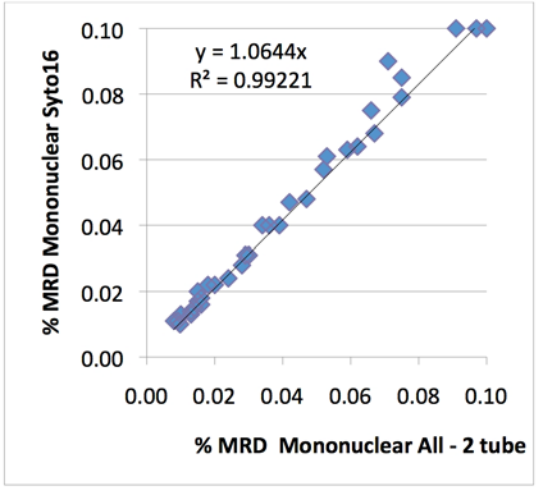
Denominator

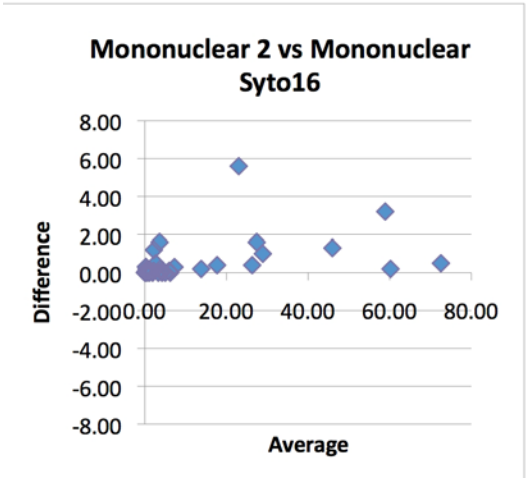


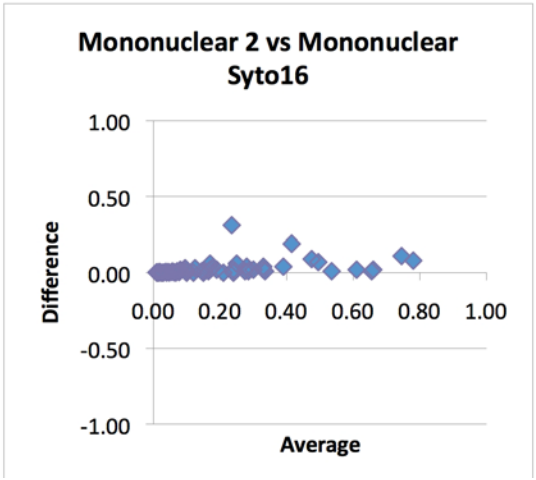
Utility of Denominator Tube

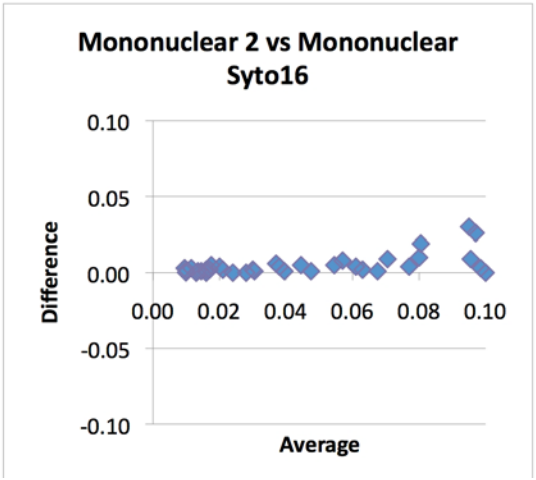












Unpublished data

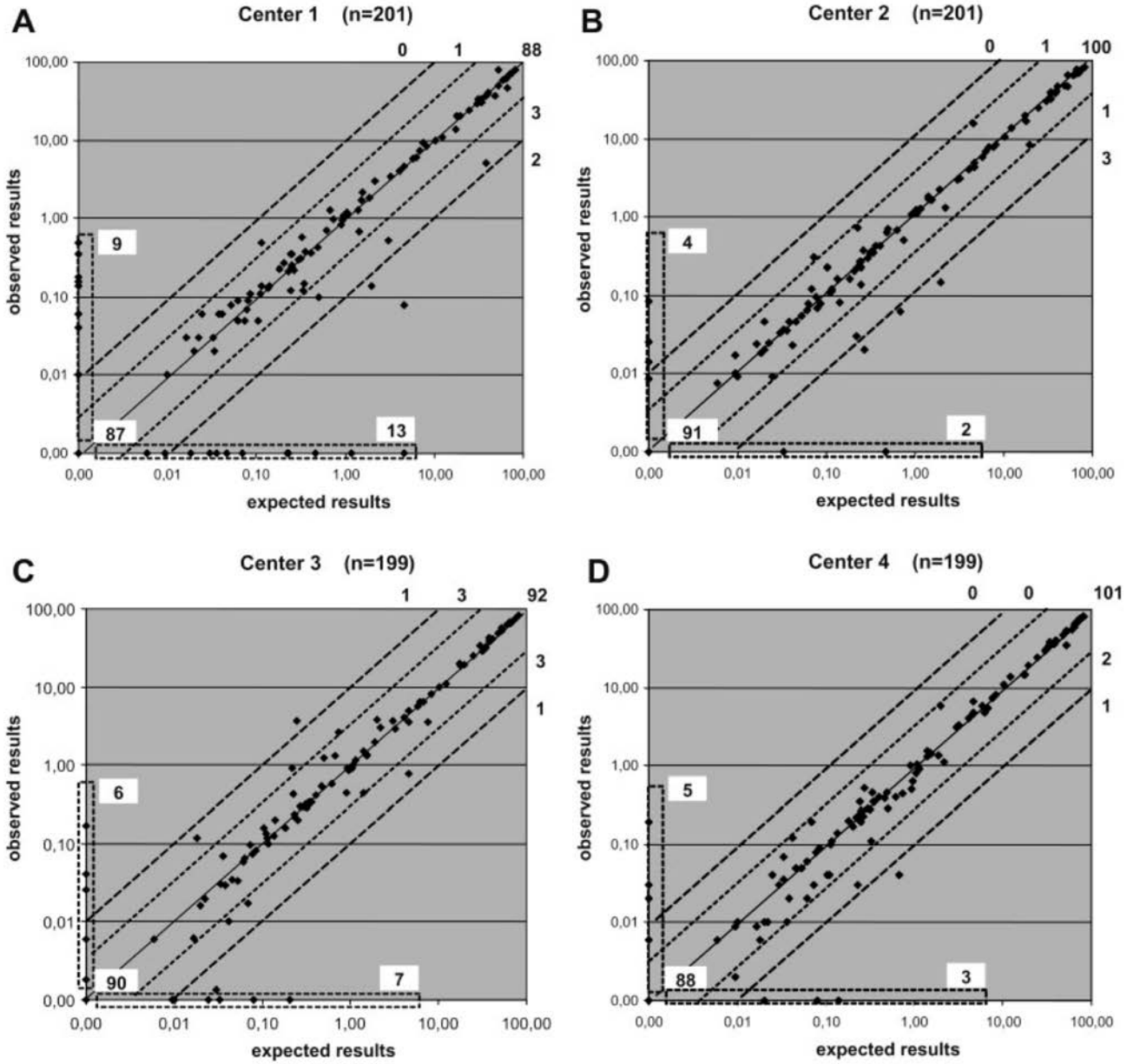
Hemodilution

- Bone marrow is a semi-solid tissue
  - Absolute cell concentration has little meaning
- Marrow aspiration is a traumatic procedure
  - Variable amount of peripheral blood introduced
  - Increased amounts of blood with each subsequent aspiration
    - 1st aspirate should be used for MRD
- Not a major problem for many samples
  - Problem in hypocellular marrows, high PB WBC count or poor quality aspirates
- No method for accurate correction
  - One method for normalization proposed for blast counts

Sources of Variability

- Identification (false positive or negative)
  - Insufficiently informative reagents
  - Improper assay validation
  - Immunophenotypic shift
  - Inexperienced interpreters
- Quantitation
  - Too few events acquired
  - Denominator effects (2 fold)
  - Sample degeneration
  - Hemodilution

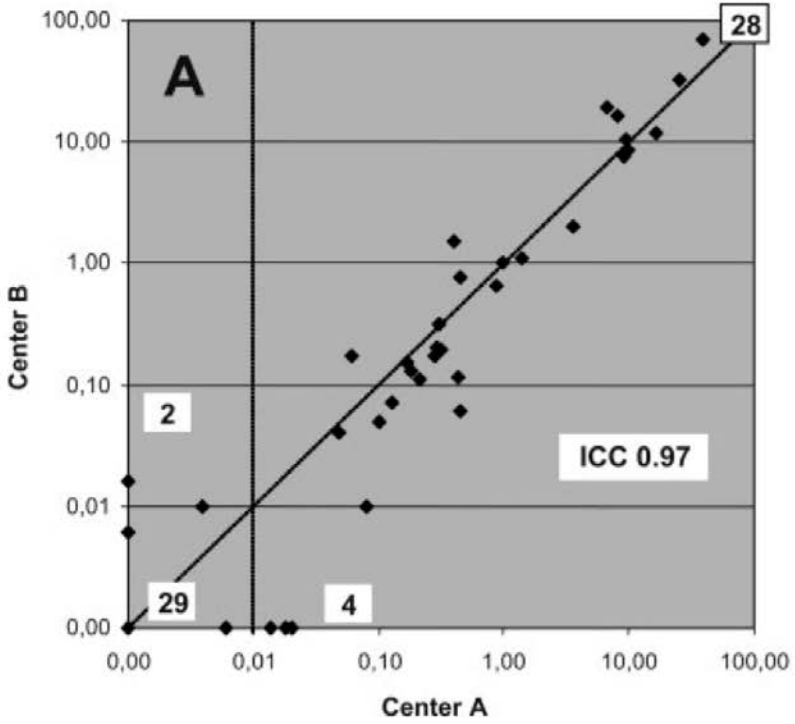
Reproducibility



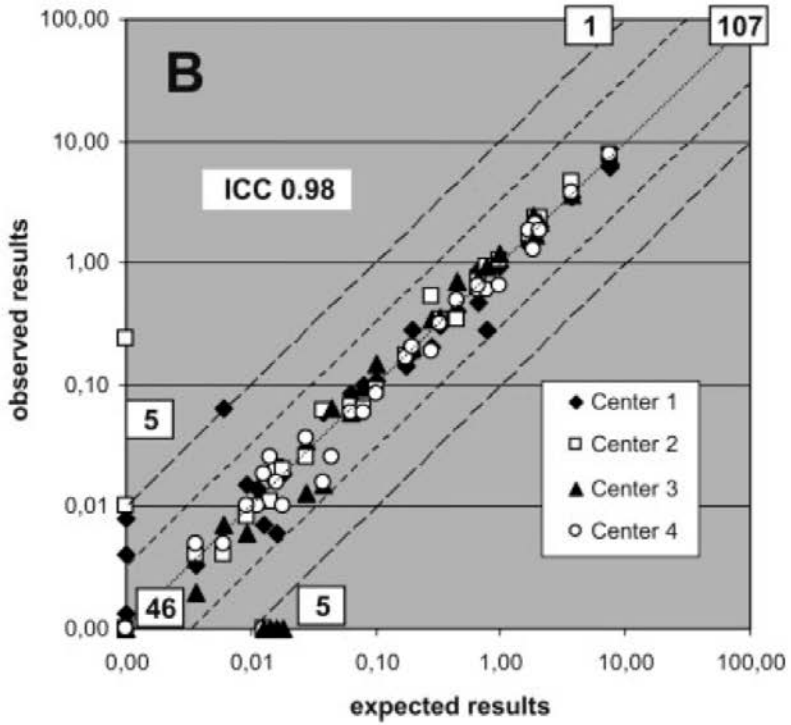
Listmode file exchange

Dworak, et al (2008) Cytometry 74B:331-340

Reproducibility



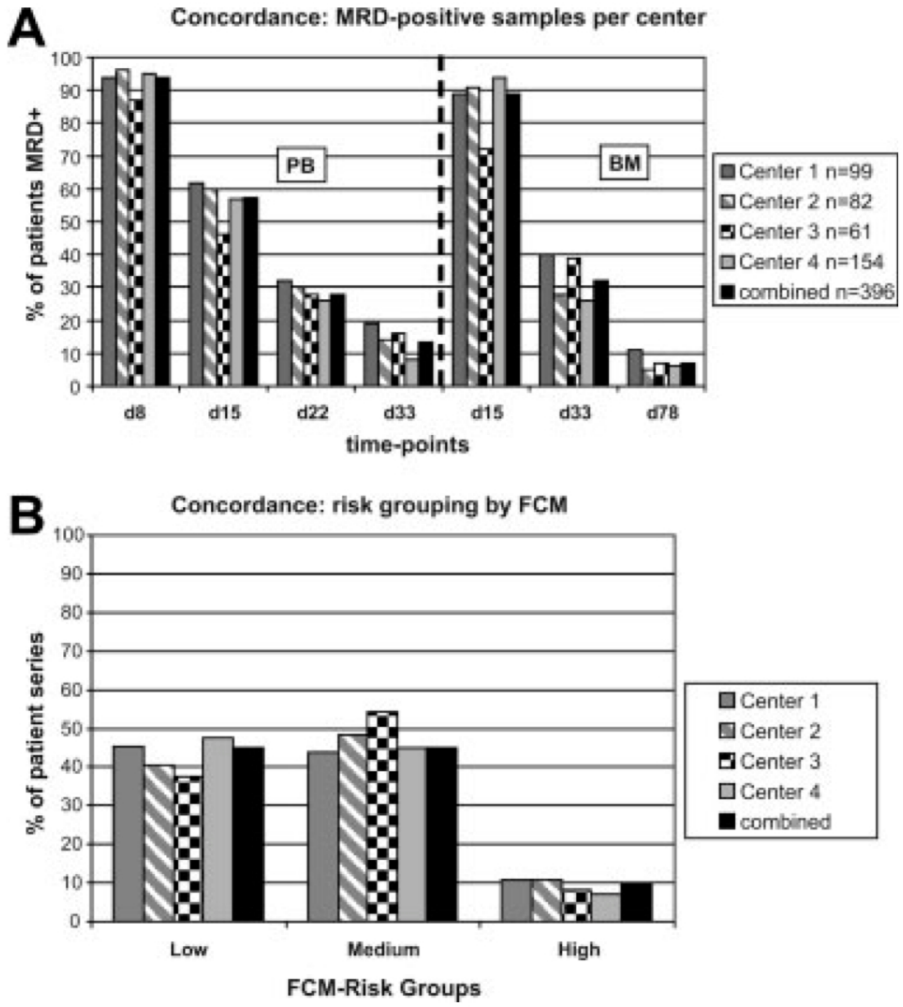
Paired sample exchange



Artificial diluted samples

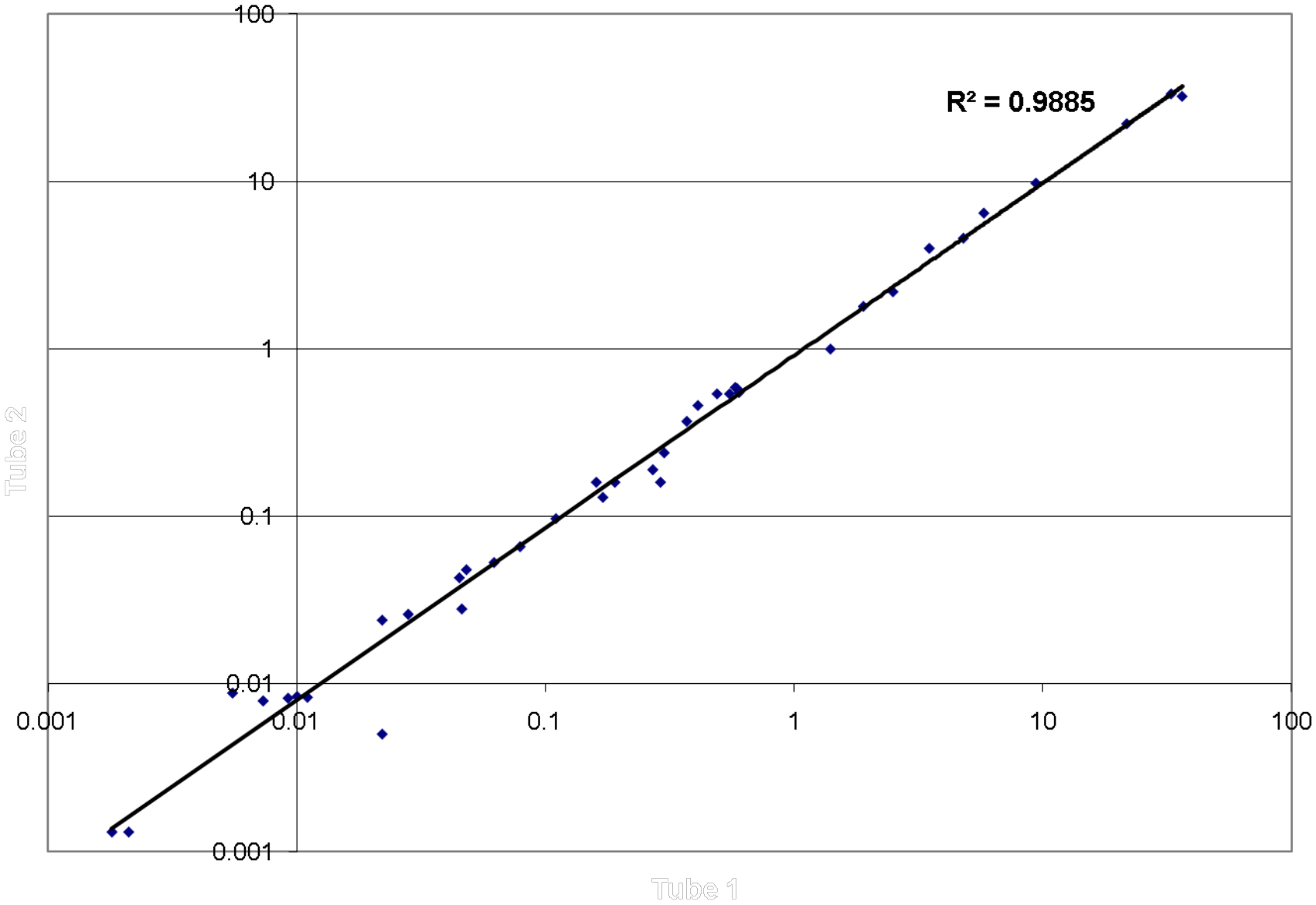
Dworak, et al (2008) Cytometry 74B:331-340

Reproducibility



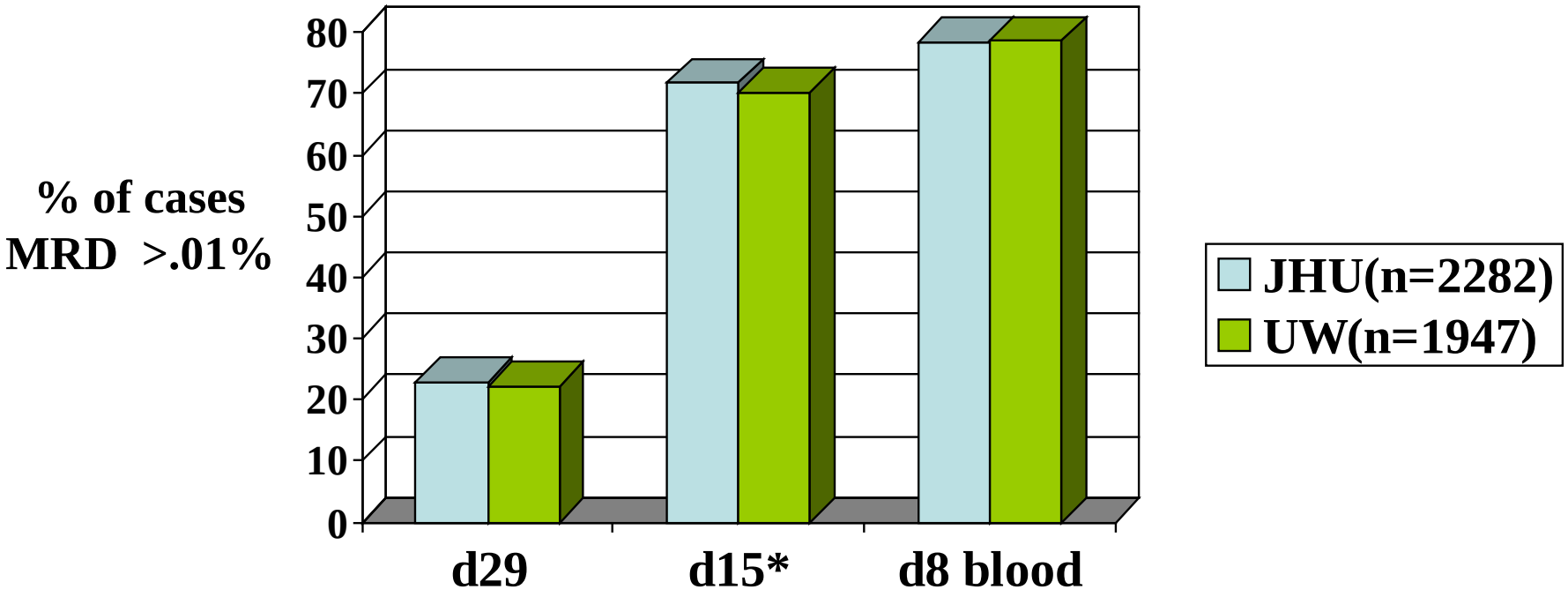
Dworak, et al (2008) Cytometry 74B:331-340

Reproducibility of MRD detection



Unpublished data, courtesy Mike Borowitz

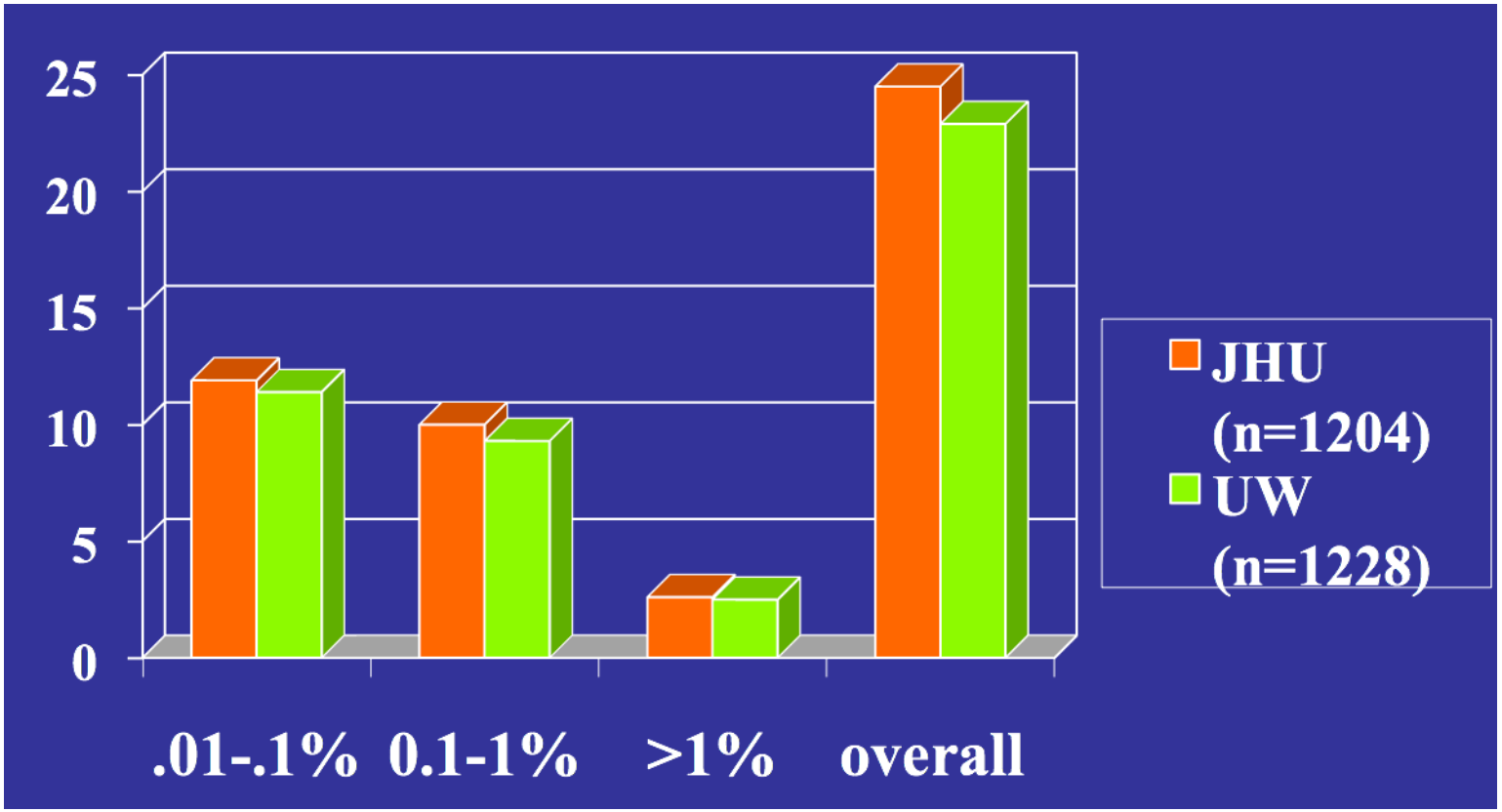
Flow MRD on AALL03B1



* day 8 M1 patients excluded

Unpublished data, courtesy Mike Borowitz

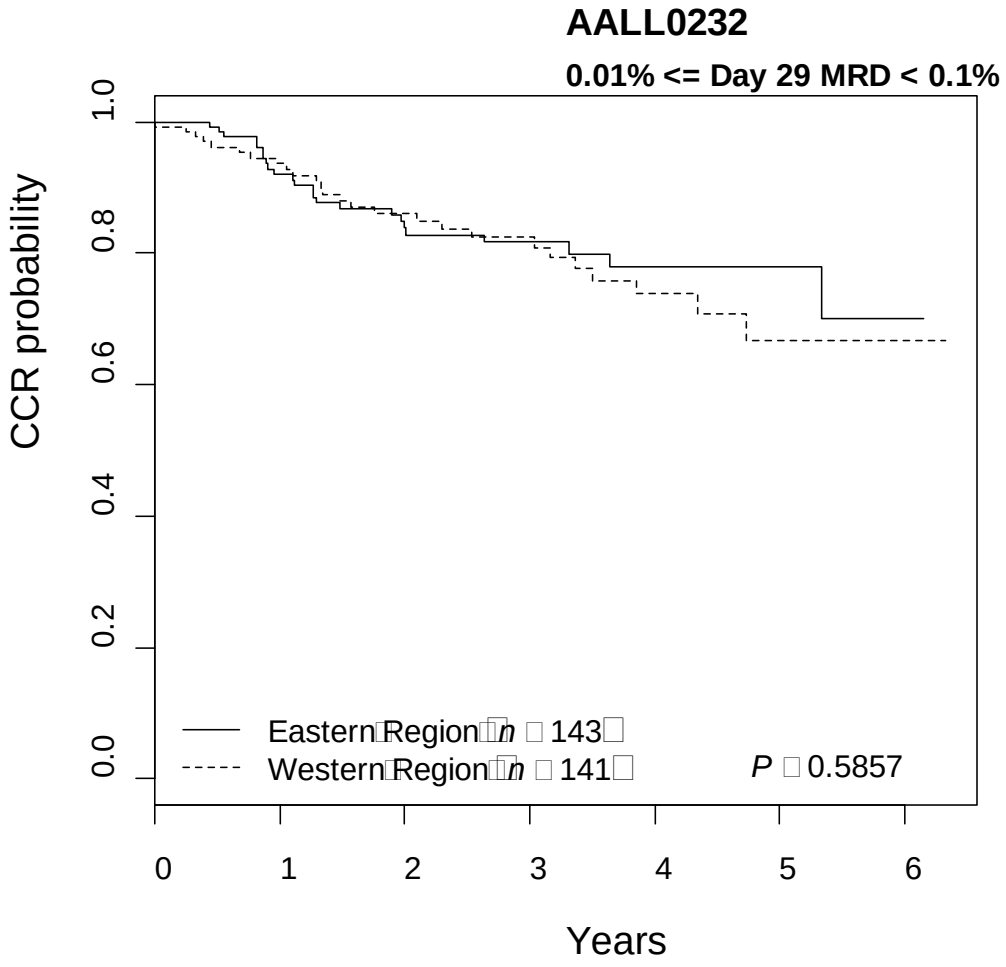
Day 29 Flow MRD on AALL0232



3/2011

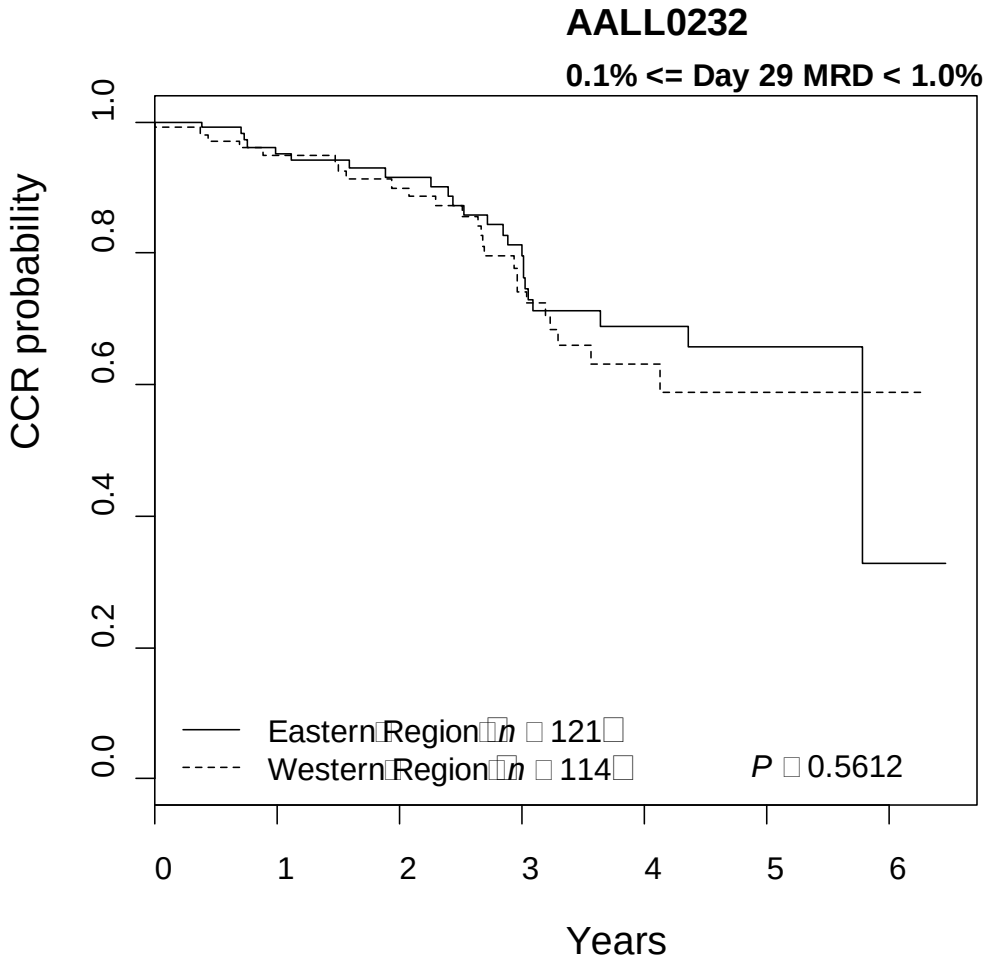
Unpublished data, courtesy Mike Borowitz

COMPARISON OF OUTCOMES
UW vs JHH MRD .01-0.1%



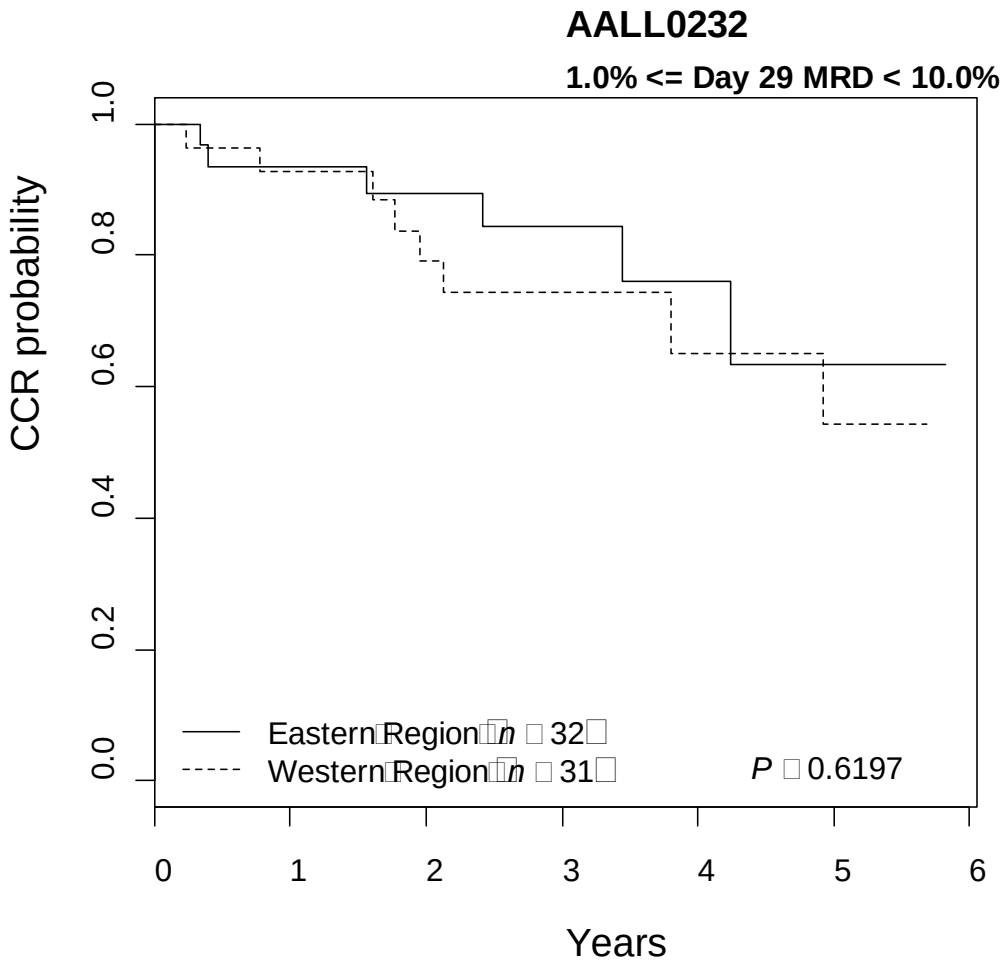
Unpublished data, courtesy Mike Borowitz

COMPARISON OF OUTCOMES
UW vs JHH MRD .1-1%



Unpublished data, courtesy Mike Borowitz

COMPARISON OF OUTCOMES
UW vs JHH MRD >1%



Unpublished data, courtesy Mike Borowitz

Conclusions

- Flow cytometry
  - Capable of accurate MRD assessment
  - Interpretative assay
  - Sensitivity
    - Dependent on
      - Antibody combination (informative)
      - Number of cells evaluated
      - Time point
    - Less than PCR (10 cells vs 1)
      - 0.01% routine for ALL
      - 0.1% routine for AML
  - Reproducible
  - Correlates with clinical outcome